THE ATTRACTIONS OF PROTEINS FOR SMALL MOLECULES AND IONS

By George Scatchard
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts

The number and variety of known compounds between proteins and small molecules are increasing rapidly and make a fascinating story. For instance, there are the compounds of iron, which is carried in our blood plasma by a globulin, two atoms of iron to each molecule of globulin held in a rather tight salt-like binding,[1] which is stored as ferric hydroxide by ferritin much as water is held by a sponge,[2] and which functions in hemoglobin, four iron atoms in tight porphyrin complexes in each protein molecule. Or, there are many compounds of serum albumin, which was used during the war by many chemists, most of whom found at least one new compound. This molecule, which has about a hundred carboxyl radicals, each of which can take on a proton, and about the same number of ammonium radicals, each of which can dissociate a proton, has one single radical which combines with mercuric ion so firmly that two albumin molecules will share one mercury atom if there are not enough to go around.[3]

At the present stage of rapid growth of known compounds, it seems more profitable for me to make no attempt to catalogue the various classes of compounds, but to discuss the general principles involved, in the hope that this will make more useful the information which is accumulating so rapidly from so many laboratories.

We want to know of each molecule or ion which can combine with a protein molecule, "How many? How tightly? Where? Why?" The answer to the first two questions, and sometimes to the third, can be furnished by the physical chemist, but he will often need to team with an organic chemist to determine the effect of altering specified groups to find if they are reactive. The determination of function is a complicated problem which may be the business of the physiologist or physiological chemist. But the answers to both of the more complicated problems will depend on the answers to the simpler questions, "How many?" and "How tightly bound?"

If the various groups on a protein molecule act independently, we can apply the law of mass action as though each group were on a separate molecule,[4] and the strength of binding can be expressed as the constant for each group. Often, a single constant will express the behavior of several groups. If the constants are widely spread, as those for the reaction of hydrogen ion with carboxylate ions, with imidazoles and with amines, the interpretation is simple. If the separation is less, it is very difficult to distinguish the case of different intrinsic affinities from the case of interaction among the groups.

We know that such interaction occurs in simple molecules in which a reaction has equal probability of happening at various points on a molecule. Reaction at one of these points may make it much more difficult for the reaction to occur at another point, as in the dibasic carboxylic acids,[5] or it may make a second reaction much easier, as in the reaction of ammonia with silver ion.[6] There may be an effect of the medium which can be interpreted by an activity coefficient, but there may also be a residue which is independent of the medium. There may be an electrostatic effect, but there may also be additional effects which cannot be explained by any simple electrostatic theory.

Independent action of the groups means that the change in free energy for the reaction of the protein with ν small molecules is made up of the statistical entropy terms plus a term proportional to ν. The simplest extension is to add another term proportional to ν^2. This extension is particularly important since it is sufficient to account for the Debye-Hückel approximation of electrostatic interaction in a medium of unchanging dielectric constant and ionic strength, or to account for non-electrostatic interaction with random distribution.

If the initial probability of reaction is the same at each of n points, the change in free energy $(\Delta F)_\nu$ for the reaction $P_0 + \nu A = PA_\nu$ is given by

$$(\Delta F)_\nu/RT = \ln c_\nu/c_0 c_A^\nu + \ln \nu!(n-\nu)!/n! - \nu \ln k + w\nu^2 \qquad (1)$$

in which RT has its usual significance, c_0, c_ν, and c_A are the concentrations of P_0, PA_ν, and A, k is the intrinsic constant for the reaction at a single group, $\nu!$ is ν factorial and w is the coefficient of ν^2. The average association $\bar{\nu} = \sum_{\nu=0}^{n} c_\nu \nu \Big/ \sum_{\nu=0}^{n} c_\nu$ is

$$\bar{\nu} = \frac{\sum_{\nu=0}^{n} \frac{n!}{\nu!(n-\nu)!} (kc_A)^\nu e^{-w\nu^2} \nu}{\sum_{\nu=0}^{n} \frac{n!}{\nu!(n-\nu)!} (kc_A)^\nu e^{-w\nu^2}} \qquad (2)$$

The calculation of $\bar{\nu}$ by this equation is straightforward, and may be extended to the case of more than one constant by addition of the respective $\bar{\nu}$'s taking into consideration the possibility that the $w\nu^2$ terms may become more complicated. This method has been used by Cannan, Kibrick, and Palmer[7] for the titration of fifty-one carboxyls, five imidazoles, and twenty-three amines in ovalbumin, and by Klotz, Walker, and Pivan[8] for twenty-two sulfathiazole groups reacting with serum albumin. If the total number of groups is large, however, the method is very tedious, and if the total number is unknown it is practically unusable.

Linderstrom-Lang[9] attempted to sum the series in the paper in which he made the first application of the Debye theory to titrations of proteins. He obtained the effect on the straight middle portion of the curve of $\bar{\nu}$ *versus* pH, but did not extend it further. Cannan, Kibrick, and Palmer[7] used the com-

plete expression. Putzeys and Bouckaert[10] derived the complete expression with very complicated mathematics. The solution for a very large number of groups is so simple and holds so well for a moderately large number of groups that it is worth while to present it free from any non-essentials.

From EQUATION 1, we find that the ratio of the concentration of all species with ν molecules of A combined with one of protein to the concentration of those with $\nu - 1$ molecules is

$$\frac{c_\nu}{c_{\nu-1}} = \frac{n + 1 - \nu}{\nu} kc_A e^{-w(2\nu-1)}. \qquad (3)$$

If the titration is to spread over only a few powers of ten, n^2w must be finite and only moderately large. Thus, $w(2\nu - 1)$ must be very small when n is large. Therefore, $c_\nu/c_{\nu-1}$ will be unity at the same value of $\bar{\nu}$ as for an ideal solution,* for which $w = 0$ and $kc = \bar{\nu}/(n - \bar{\nu})$, and $\nu = \bar{\nu} + \bar{\nu}/n$: substituting this value in EQUATION 3 and transposing yields

$$ke^w c_A = \frac{\bar{\nu}}{n - \bar{\nu}} e^{2(1+1/n)w\bar{\nu}}$$

$$k'c_A = \frac{\bar{\nu}}{n - \bar{\nu}} e^{2w'\bar{\nu}} \qquad \ln k'c = \ln \frac{\bar{\nu}}{n - \bar{\nu}} + 2w'\bar{\nu} \qquad (4)$$

in which $k' = ke^w$ and $w' = \left(1 + \frac{1}{n}\right)w$. Rather than trying to prove that this is the limiting expression for very large values of n, let us see how bad it is for very small values.

We find that titration curves often have shapes not unlike that for $w = 0$, which is shown as curve 4 in FIGURE 1, except that they are spread about

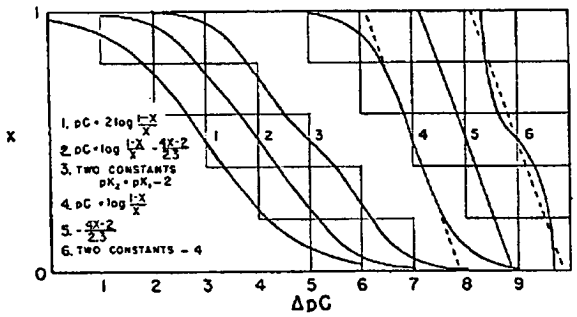

FIGURE 1. Titration Curves.

twice as far on the pH axis. How can the ideal curve be warped to give this spread? Although the central portion of the curve is nearly linear, it is not

* We note that, if w is so large that reactions at different points are completely separated, so that $PA_{\nu-1}$ and PA_ν are the only forms of the protein present, $\nu = \bar{\nu} + \frac{1}{2}$. The difference, $\bar{\nu}/n - \frac{1}{2}$, is to be compared to n. Moreover, the correct value is much nearer $\bar{\nu}/n$ than to $\frac{1}{2}$ for any probable values of w.

permissible to rotate the curve about its center because the asymptotes must remain horizontal with $x = 0$ and $x = 1$. If the reactions at half the points have an intrinsic dissociation constant different from those at the other half, we divide curve 4 into two equal parts, pull them apart horizontally, and then add. Curve 3 shows the result if k'' is one-hundredth of k'. If EQUATION 4 is valid, each point should be displaced horizontally by an amount proportional to its perpendicular distance from the midpoint. Curve 2 corresponds to EQUATION 4 with $w'n = 2$. It is also possible to displace each point by an amount proportional to its horizontal distance from the midpoint, which is equivalent to changing the scale of abscissae. In curve 1, each point has double the horizontal displacement of curve 4. When the displacement of curve 4 is divided by an integer, the resulting curve corresponds to reaction of that integral number of molecules with one protein without any intermediate forms. I can find no physical explanation for this type of curve for displacement greater than that of curve 4, but I have included it because this expression is often used. Curves 4, 5, and 6 represent the difference in horizontal displacement of curves 1, 2, and 3 from 4, and the broken lines with 4 and 6 are repetitions of 5.

FIGURE 2 shows the differences in $\bar{\nu}/n$ of curve 3 of FIGURE 1 from curve 2

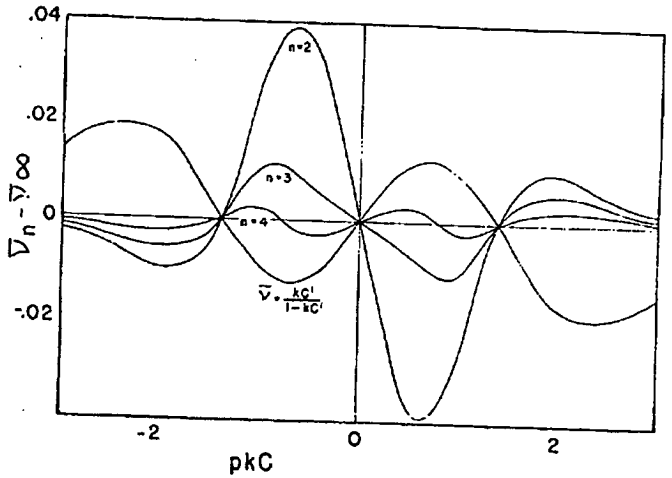

FIGURE 2. Effect of Finite Number of Groups. Deviations from $pkC = \log \frac{1-x}{x} - \frac{4x-2}{2.3}$.

at the same value of pkc. The maximum difference is 3.9 percent, and the difference is zero when kc is 0.04, 1, or 25. FIGURE 2 also shows the difference from curve 2 of a curve of the type of curve 1, and of the curves for $n = 3$ and $n = 4$ which intersect curve 2 at these same points as curve 3. For three groups, the maximum deviation is 1.2 percent; for four groups it is 0.3 percent, and the deviation is also zero for $kc = 0.1$ and 10. Thus, four is

practically infinity within the accuracy of most measurements if the curve is spread twice the width of curve 4, FIGURE 1.

For the Debye-Hückel approximation for a charge spread uniformly over the surface of a sphere of radius b which excludes small ions to a radius a,

$$w = \frac{\epsilon^2 z^2}{2DkT}\left(\frac{1}{b} - \frac{\kappa}{1 + \kappa a}\right) \qquad (5)^{*,11}$$

in which D is the dielectric constant of the medium, k is Boltzmann's constant, T the absolute temperature, ϵ the electronic charge, z the valence of the small molecule, and κ has its usual significance in the Debye theory, and the net valence of the protein replaces ν in $w\nu^2$.

The expression for a discrete distribution of charges within a spherical molecule is given by Kirkwood,[12] as modified by Kirkwood and Westheimer.[13] The greatest difference from the Debye-Hückel expression is that b is a function of the charge distribution and dielectric constant of the protein molecule, and is such a function of the dielectric constant of the medium that $1/Db$ does not vanish for an infinite dielectric constant. Kirkwood's expression also gives a more complicated variation with ionic strength, but this will be so smeared by non-electrostatic effects that, in the present state of our knowledge, it will be sufficient to use this equation with the conditions that b need not be too closely related to the size of the molecule, and a is always greater than b. These expressions are all limited to very small protein concentrations. The effect of increasing concentration is discussed by Scatchard, Batchelder, and Brown.[14] Usually we do not know enough about the protein to justify considering second approximations.

At times, I have been troubled by the fact that the probability of reaction is not the same for all points once reaction has occurred at one of them. I know that I have not been alone in this worry, so it has seemed worth while to consider the electrostatic effects for two simple distributions: a regular tetrahedron and a cube at zero ionic strength in a medium of the same dielectric constant as the large molecule. The results are shown in FIGURE 3. Putting on the first small molecule will require no electrostatic work, but for each additional one there will be work proportional to the sum of the reciprocal distances to each charge already there.

For the regular tetrahedron, the distances are all the same and there is one form with no charge, four with one charge, six with two, four with three, and one with four charges. The works are proportional to 0, 1.7, 5.2, 10.3, or to 0.86 $\nu(\nu - 1)$. The works are normalized so that the work for the fully charged cube is $\nu(\nu - 1)$. For the smaller charges, the number of each form and the work is listed below the model of the form. The differences from $\nu(\nu - 1)$ are so small for most of the molecules that my worries have been dissipated.

* The limit at very small values of κ is $(\epsilon^2 z^2/2DkT)/b$, and at very large values of κ it is $(\epsilon^2 z^2/2DkT)(1/b - 1/a)$. Two parameters, a and b, are necessary to keep the latter limit different from zero.

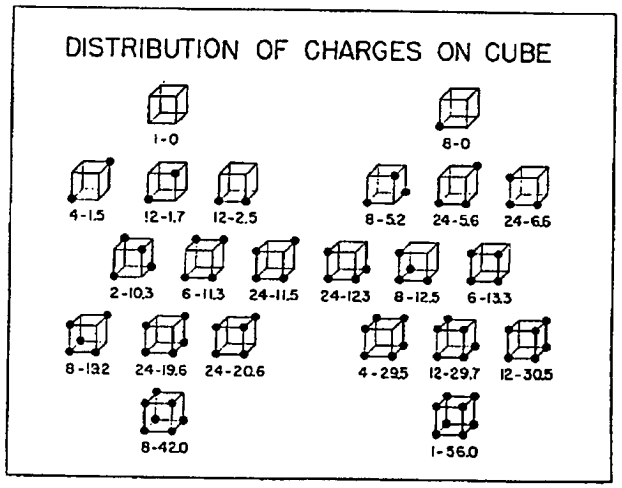

FIGURE 3.

There seems to be but little known of negative values of w for protein compounds. However, we might expect cases when the van der Waal's attraction of long chains should overcompensate electrostatic repulsion just as it does in soap micelles. When nw equals -2, the slope of $\bar{\nu}$ *vs.* $\log c_A$ becomes infinite, and for larger values there is a sudden jump from small values to large. The electrophoresis of albumin and decanyl sulfate by Putnam and Neurath[15] probably indicates an effect of this kind. The first compound may represent saturation with charged ends toward the protein, while the second may have a reversed layer, giving a soap micelle wrapped around the protein molecule.[16]

FIGURE 4 shows the titration of ovalbumin with HCl or KOH in the presence of KCl in various amounts by Cannan, Kibrick, and Palmer.[7] The

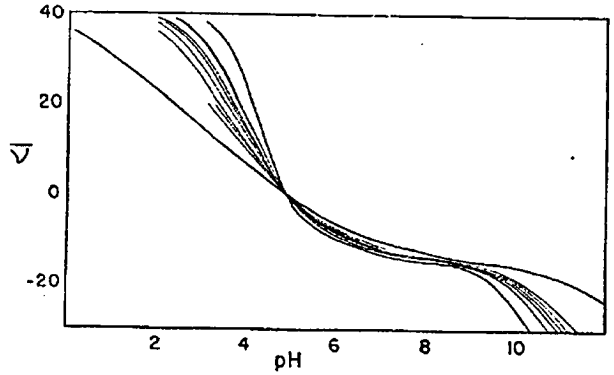

FIGURE 4. Titration of Ovalbumin.[7]

steepest curve is the ideal curve they plot for 51 groups with pk = 4.29, 5 with pk = 6.7, and 23 with pk = 10.07. The flattest curve is the one they calculate for zero ionic strength. The intermediate curves are those for their titrations at various ionic strengths, displaced so that they all intersect at the isoionic point. The corrections for activity coefficients are in the opposite direction from those to the ideal titration curve.

FIGURE 5 shows the same measurements represented as ΔpH, the pH

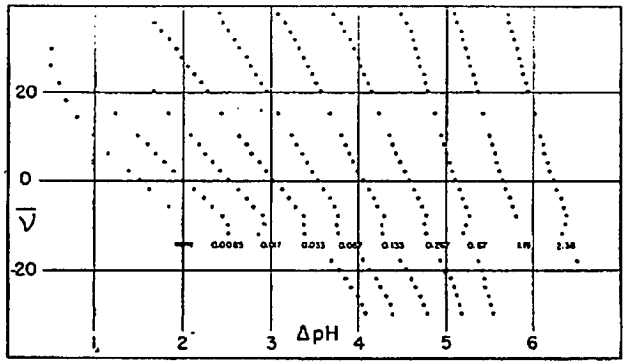

FIGURE 5. Titration of Ovalbumin.[7]

minus that of the ideal solution. Although the tables have been smoothed, I have preferred not to draw curves. For example, the quirks near $\bar{\nu} = -15$ may well be introduced by smoothing with respect to $\bar{\nu}$ and not to pH in poorly buffered regions. Here, the displacement of the isoionic point is shown with a maximum of 0.22 units. The curves are nearly linear. The left-hand curve is for no added salt. At the bottom it is very flat, but it rises steeply and even slopes backward as the decrease in electrostatic effects with increasing ionic strength more than compensates for the increased charge. If EQUATION 5 is valid, exact compensation is reached when

$$\frac{\kappa(1 + \kappa a) + \bar{\nu} d\kappa/d\bar{\nu}}{(1 + \kappa a)^2} = \frac{1}{b}.$$

There is an additional effect due to the displacement of the isoionic point on the addition of electrolyte.

FIGURE 6 shows the titration of wool with hydrochloric acid in the presence of various amounts of KCl by Steinhardt and Harris,[17,18] with the ideal pH for 0.83 millimoles per gram maximum deducted. These points represent the unsmoothed experimental measurements. The points are represented approximately by straight lines, but the approximation is apparently not within the experimental accuracy. Reasons for this discrepancy must wait upon a more detailed study. Again, the left-hand curve shows the titration with no added salt, which has about the same shape as that in FIGURE 5. The displacement of the isoionic point is greater for wool than for ovalbumin,

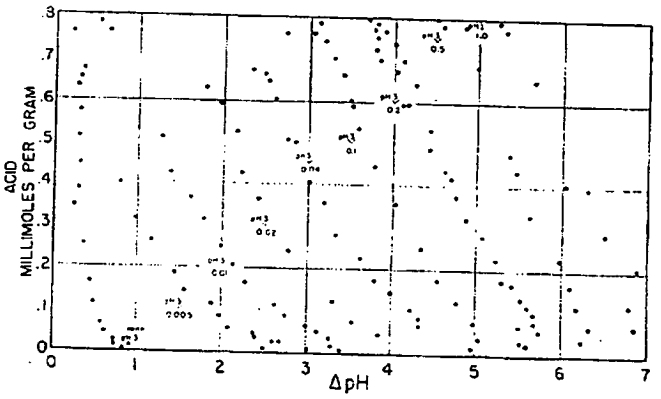

FIGURE 6. Titration of Wool with HCl.[17]

though it is harder to see through the scatter of the points at low titer. The authors attribute this displacement to combination of the anions with the wool and have calculated the association constants for many anions from the titration curves of the corresponding acids.[19] They find that the affinity increases rapidly with the molecular weight.

My own experience with combinations of protein with molecules other than hydrogen or hydroxyl ion came with our measurements of the osmotic pressure of serum albumin in solutions of sodium chloride.[14] The salt distribution practically forced us to assume such a combination. Making the naive assumption that this combination is responsible for all the difference in salt concentration outside and inside the membrane leads to the extent of combination shown in FIGURE 7 for the values of the valence and pH shown on the abscissae. The points shown are for NaCl concentrations about 0.15 M. We found no more combined at 0.2 M and no less at 0.05.

It is amusing to see if an equally naive picture will explain the deviation of osmotic pressure from the ideal solution laws. The open circles show the

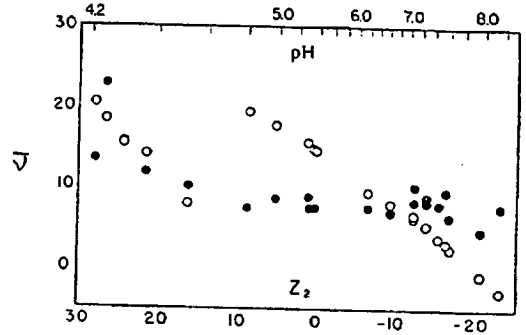

FIGURE 7. Serum Albumin and Chloride Ion.[14]

extent of combination necessary to explain these deviations as pure Donnan effects. The value of $\bar{\nu}$ falls regularly as the valence becomes less positive, jumps suddenly near the isoionic point, and then falls again steadily. A glimpse at FIGURE 8 will explain the behavior. The points are B from the equation

$$PV^\circ = RTm_2(1 + B\overline{W}_2m_2)$$

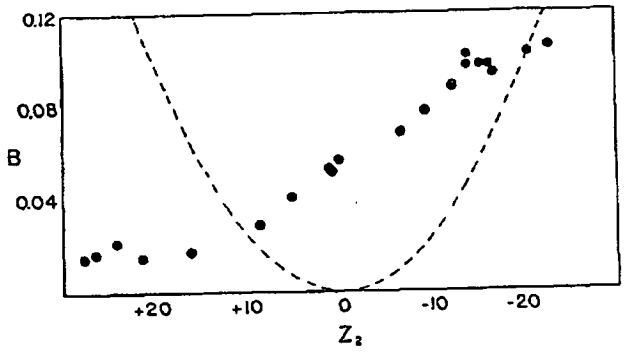

FIGURE 8. Serum Albumin and Chloride Ion.[14]

in which P is the osmotic pressure, V° the volume containing a kilogram of water, m_2 the molal concentration of protein, and $\overline{W}_2$ its molecular weight. The broken line is the Donnan value. The extent of combination calculated in FIGURE 7 is the horizontal displacement necessary to bring the point to the Donnan line, and the break near the isoionic point separates those points which are moved to the right branch from those which are moved to the left. Taking into account the effect of the combination indicated by the salt distribution makes the effect of albumin on its own activity coefficient more symmetrical, but it does not reduce much the maximum effect, which must be taken into account.

Recently, we have been studying the combination of chloride ion and of thiocyanate ion with human serum albumin by a procedure like that of Klotz,[8] except that the concentration is determined by conductance. This necessitates measurements without added salt and therefore at varying ionic strength. The experimental results will be reported in a later paper, but I want to discuss here the method of treatment which we developed for them.

For the acid titrations, the maximum binding capacity can be approached closely with relatively low concentrations of acid or base. For the weaker associations, which we are considering now, the average amount bound is still increasing rapidly when only a small fraction of the small molecules are combined. This makes the determination of the maximum binding less certain, and our task is to reduce that uncertainty as far as possible.

Recent usage has been to invert the law of mass action solved for $\bar{\nu}$ to give

$$\frac{1}{\bar{\nu}} = \frac{1 + kc}{knc} = \frac{1}{n} + \frac{1}{knc} \tag{6}$$

to plot $1/\bar{\nu}$ against $1/c$, to draw the best straight line and call its intercept $1/n$ and its slope $1/kn$. This has the disadvantage of concealing deviations from the ideal laws, and of tempting straight lines where there should be curvature.

I have preferred to start with the mass action law solved for c:

$$\bar{\nu}/(n - \bar{\nu}) = kc$$

and multiply by $(n - \bar{\nu})/c$ to give

$$\bar{\nu}/c = k(n - \bar{\nu}). \tag{7}$$

Plotting $\bar{\nu}/c$ against $\bar{\nu}$ again gives a straight line if k is constant. The intercept on the $\bar{\nu}/c$ axis is kn, the classical first association constant, and the intercept on the $\bar{\nu}$ axis is n. This plot shows immediately how great is the extrapolation necessary to determine these quantities.

Curvature may indicate different intrinsic constants or deviations from independent probabilities. In the latter case, we may alter EQUATION 4 to give

$$\bar{\nu}e^{2w'\bar{\nu}}/c = k'(n - \bar{\nu}) \tag{8}$$

and plot $\bar{\nu}e^{2w'\bar{\nu}}/c$ against $\bar{\nu}$. Sometimes w' may be calculated theoretically, or an approximate value may be determined empirically. It is not necessary to straighten the line if the correction is good enough to determine the intercepts. Even if there are different intrinsic constants, the two intercepts are still the classical first association constant and the total number of groups.

As an example, we show the titration of ovalbumin of Cannan, Kibrick, and Palmer[7] with 32 g. protein per liter and no added salt. In FIGURE 9, the

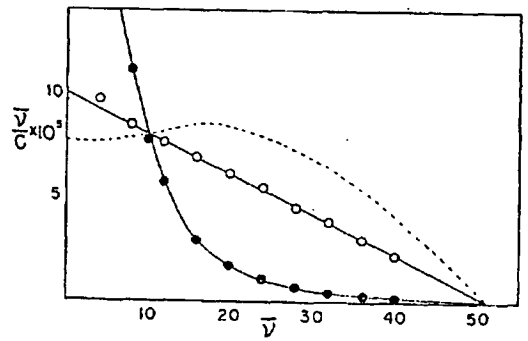

FIGURE 9. Titration of Ovalbumin.[7]

full circles are the measured values of $\bar{\nu}$ assuming that $\bar{\nu}$ is ten for the isoionic protein. The open circles are corrected by the equation corresponding to

the authors' assumption that the correction is 0.8, the Debye value for b = 27.5 Å and a = 29.5 Å, which gives

$$2w/2.3 = 0.084[1.073 - 9.68\sqrt{\mu}/(1 + 9.68\sqrt{\mu})]$$

in which μ is the ionic strength. The straight line corresponds to their values of $n = 51$ (obtained from special experiments) and $\log k = 4.29$. The curve is obtained from this line by making the correction in reverse. The measured value for the most dilute point falls off the scale. The corrected value begins to show the effect of the imidazole groups, which are not counted in $\bar{\nu}$.* The broken line shows the effect of correcting the lower curve by the full Debye value for the dimensions chosen by the authors, that is, with w 1.25 times that for the straight line. Although it is obviously overcorrected, the values of $(n - \bar{\nu})$ and of $k\bar{\nu}$ at the isoionic point could be obtained from it with very fair accuracy.

FIGURE 10 shows the results of Klotz, Walker, and Pivan[6] on bovine serum

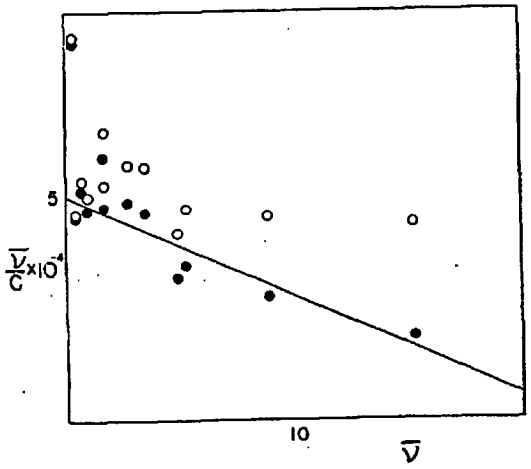

FIGURE 10. Serum Albumin and Methyl Orange.[6]

albumin and methyl orange. Again, the filled circles are the measured values and the open circles are corrected for electrostatic effects using the dimensions which the authors used for sulfathiazole. The straight line is that determined by the values of n and k given by the authors. It is not certain that these measurements should be corrected for electrostatic effects. They are made in 0.1 M phosphate buffer, and it is possible that the methyl orange replaces phosphate ion instead of reacting with uncombined albumin. The extrapolation of these results is much less certain that that in FIGURE 9.

FIGURE 11 shows their results for serum albumin and sulfathiazole. The correction is the same as that made by the authors and the straight line is

* The correction is made for $\bar{\nu} - 10$, so that there is none at the isoionic point, and the value of k should be determined as $\bar{\nu}/c(n - \bar{\nu})$ for the isoionic point.

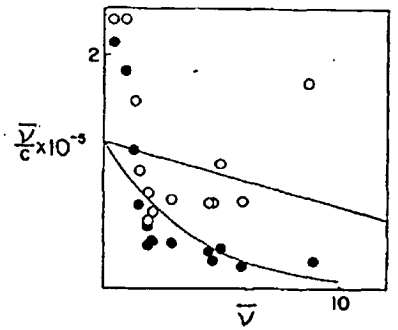

FIGURE 11. Serum Albumin and Azosulfathiazol.[6]

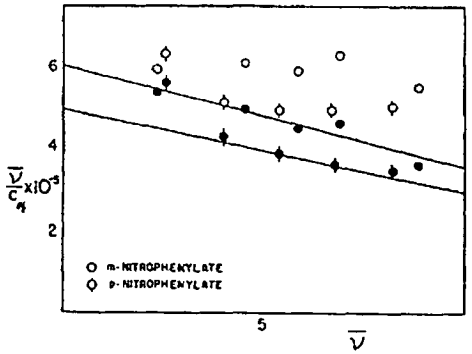

FIGURE 12. Serum Albumin and m- and p-Phenylate.[20]

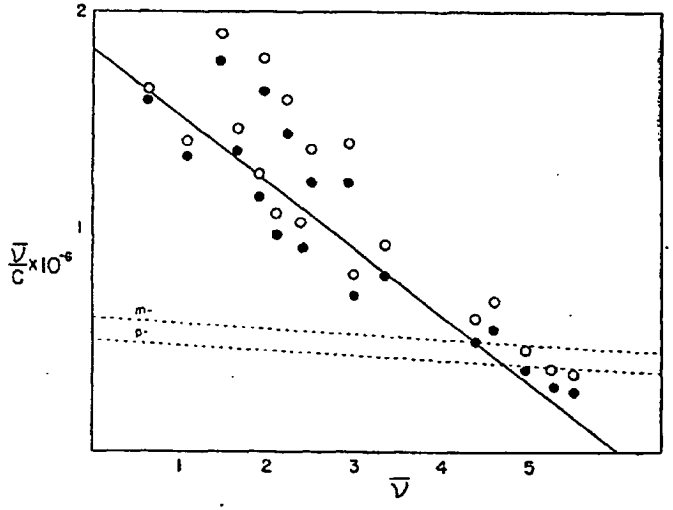

FIGURE 13. Serum Albumin and o-Nitrophenylate.[20]

determined from their values of n and k. The curve through the experimental points is made by applying the correction in reverse to the straight

line. If the reaction is the displacement of a univalent ion by a bivalent one, the correction should be only the fourth root of that used.

FIGURES 12 and 13 show the results of Teresi and Luck[20] for bovine serum albumin and some nitrophenolates. Again, the filled circles represent the measured values, the open circles are corrected for electrostatic effects as in FIGURE 10, and the lines are determined from the values of k and n given by the authors. These reactions may also be displacements of buffer anions so the electrostatic correction may be improper.

Although the precision of extrapolation is not very great, the results are quite sufficient to show the difference between the orthophenolate on one hand and the meta- and paraphenolates on the other. For the orthophenolate, k is much larger and n much smaller. The authors find smaller values of n for many orthonitrophenols, and they attribute the difference to steric hindrance. It is not surprising that an ortho-nitro group favors association at points where the steric hindrance does not interfere.

Much of the difference between the precision of extrapolation in FIGURE 9 for hydrogen ion and in the subsequent figures for anions depends upon the much greater magnitude of the association constants for the acid titration. However, these figures should show the great importance of the greatest possible precision over the widest possible range in order that these curves may be extrapolated accurately to the intercepts which tell us "how many" and "how tightly bound."

Bibliography

1. SORGENOR, D. M., B. A. KOECHLIN, & L. E. STRONG. J. Clin. Inv. In press.
2. MICHAELIS, L. 1947. Advances in Protein Chemistry **3**: 53.
3. HUGHES, W. L. 1947. J. Am. Chem. Soc. **69**: 1836.
4. VON MURALT, A. L. 1930. J. Am. Chem. Soc. **52**: 3518.
5. HAMMETT, L. P. 1940. *Physical Organic Chemistry:* 201. McGraw Hill. New York.
6. BJERRUM, N. 1941. *Metal Amine Formation in Aqueous Solution.* P. Haase and Son. Copenhagen.
7. CANNAN, R. K., A. KIBRICK, & A. H. PALMER. 1941. Ann. N. Y. Acad. Sci. **41**: 243.
   1942. Chem. Rev. **30**: 395.
8. KLOTZ, I. M., F. M. WALKER, & R. B. PIVAN. 1946. J. Am. Chem. Soc. **68**: 1486.
9. LINDERSTROM-LANG, K. 1924. C. R. Lab. Carlsberg **15** (7).
10. PUTZEYS, P., & L. BOUCKAERT. 1942. Medd. Kon. Vlaamsche A. Wet., L. & S. K., van Belgie **4** (3).
11. SCATCHARD, G. 1927. Trans. Faraday Soc. **23**: 454.
12. KIRKWOOD, J. G. 1934. J. Chem. Phys. **2**: 351.
13. KIRKWOOD, J. G., & F. H. WESTHEIMER. 1938. J. Chem. Phys. **6**: 506.
14. SCATCHARD, G., A. C. BATCHELDER, & A. BROWN. 1946. J. Am. Chem. Soc. **68**: 2320.
15. PUTNAM, F. W., & H. NEURATH. 1944. J. Am. Chem. Soc. **66**: 1992.
16. PARKHURST, K. G. A., & R. C. N. SMITH. 1944. Trans. Faraday Soc. **40**: 565.
17. STEINHARDT, J., & M. HARRIS. 1940. J. Res. N. B. S. **24**: 335.
18. STEINHARDT, J. 1941. Ann. N. Y. Acad. Sci. **41**: 287.
19. STEINHARDT, J., C. H. FUGITT, & M. HARRIS. 1942. J. Res. N. B. S. **28**: 201.
20. TERESI, J. D., & J. M. LUCK. 1948. J. Biol. Chem. **173**: 653.

DETERMINATION OF THE DISSOCIATION CONSTANTS OF WEAK ELECTROLYTES IN SALT SOLUTIONS

BY MARTIN KILPATRICK

Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois

In connection with kinetic studies, it is often essential to have a fairly accurate knowledge of the hydrogen ion concentration of aqueous and non-aqueous solutions in the presence of appreciable concentrations of electrolytes. There now exist precise values of the thermodynamic dissociation constants of a number of carboxylic acids in aqueous solution, and in some cases the dissociation constants are known over a considerable range of concentration for a few electrolytes. These dissociation constants have been determined by five general methods: (1) conductance; (2) electromotive force methods; (3) colorimetry; (4) kinetic measurements; and (5) solubility.

It is the purpose of this paper to review the use of the kinetic method for determining the dissociation constants of monobasic acids and to point out the difficulties and assumptions involved when the concentration of electrolytes is high.

In the early days of the Arrhenius theory, Ostwald and others used the kinetic method to obtain the ratio of the dissociation constants of acids. Essentially, the method consisted in obtaining the velocity constant for the inversion of sucrose or the hydrolysis of methyl acetate in solutions containing equivalent concentrations of the acids in question. The ratio of the velocity constants was taken as the ratio of the dissociation constants. These results were often combined with dissociation constants obtained from conductance calculations based on the assumption that $\Lambda/\Lambda_\infty = \alpha$ and the Ostwald Dilution Law. For example, Ostwald[1] determined the dissociation constant of dichloroacetic acid from conductance measurements at 25° C. as 0.0514 and then determined the ratio of this dissociation constant to that of trichloroacetic acid by a comparison of the rates of inversion of sucrose for the two acids and reported a value of 1.2 for the dissociation constant of trichloroacetic acid. We shall see later that there is no reliable value of the dissociation constant of trichloroacetic acid in the literature at the present time. In using the kinetic method to determine hydrogen ion concentration, Ostwald assumed that the rate of reaction was directly proportional to the hydrogen ion concentration and neglected any electrolyte effects on the rate of reaction.

With the introduction of empirical rules for activity coefficients[2,3] and of the Debye-Hückel equation,[4] together with the solution of the conductance problem,[5] many of the difficulties of methods 1, 2, 3 and 5 for determining